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Research Article

Sustainable Valorization of Amazonian Pracaxi (*Pentaclethra macroloba* (Willd.)) Residues via Optimized Ultrasound-Assisted Extraction of Bioactive Compounds

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Abstract

Pracaxi is an Amazon oil very appreciated by industries due to its biological characteristics, when it is processed to generate a biomass that is generally accumulated without proper disposal. This material is a source of phytochemicals with high biological activity. The Design of Experiments (DOE) is an interesting approach to optimize processes capable of controlling variables that influence this process, without the need to perform many tests. The aim of this work was to optimize the ultrasound-assisted extraction of bioactive compounds (Total phenolic compounds -TPC, Total flavonoids- TF and antioxidant activity - AA) from pracaxi biomass residue through an axial 2^3 experimental design, with desirability tool and Response Surface Methodology (RSM), the effects studied were: extraction time in ultrasound (min) (x1), solid-liquid ratio (% m/v) (x2) and ethanol concentration (% v/v) (x3), the dependent variables were TPC, TF and AA. The experimental design showed that variables with the most significant effect in this study were the x2 followed by the x3 and x1. The theoretical optimal condition was achieve using desirability tool with

overall value of D= 63.58% with the following conditions: 0.738 mg GAE/g for TPC, 0.023 mg QE/g and 100 µmol ET/g of sample. The experimental values obtained achieved error values of 13.17%; 17.85% and 8.78%, respectively. Indicating that the model obtained can be considered satisfactory. Using ethanol with assisted ultrasound is an efficient technique for the extraction of bioactive compounds from pracaxi, being economically viable giving the possibility of valuing a residue from the Amazon, contributing to the circular economy.

Keywords

Amazon oilseeds; experimental design; optimization; extraction process; ultrasound-assisted process.

Highlights

- Ultrasound-assisted extraction was optimized for bioactives from pracaxi residues.
- Solid–liquid ratio was the most influential factor in extraction efficiency.
- The process used ethanol, a green solvent, aligning with sustainable practices.
- The method adds value to Amazonian biomass and supports circular bioeconomy.

Introduction

The Brazilian Amazon is very rich in its biodiversity. Among the potential raw materials are forest extraction products, such as andiroba, copaiba and pracaxi (*Pentaclethra macroloba* (Willd.)) oils, and this last has gained space in the pharmaceutical and cosmetics industries, with potential in the dermatological and hair sectors [1,2].

Pentaclethra macroloba (Willd.) belongs to the Fabaceae family [3]. This oilseed is common in the Amazon region, with trees reaching heights of up to 14 meters and diameters of up to 59 cm [4]. Its fruits exhibit dark green coloration, however, during their ripening process, their coloration changes to a more intense green [5].

Pracaxi oil is one of the most appreciated products by the pharmaceutical and cosmetic industry due to its biological characteristics. Its composition includes several fatty acids, including 53% oleic acid and 16% behenic acid, as well as linoleic and lignoceric acids [6], [7].

Oilseeds processed in industries in the food, cosmetics, pharmaceutical and biodiesel sectors generate by-products and processing waste that are generally accumulated without proper disposal. In general, this waste is used to produce organic fertilizers or for animal feed [8].

Phytochemicals, found naturally in plants, have beneficial effects on human health, such as phenolic acids (gallic, hydroxybenzoic, caffeic, coumaric, ferulic and ellagic) and their derivatives, and flavonoids (catechin, epicatechin, myricetin, quercetin and kaempferol), these compounds being of interest mainly to the chemical and pharmaceutical industry [9].

The phytonutrient sector, which includes these compounds, generated approximately 5.2 billion dollars in 2018 and is expected to grow 7.3% annually until 2024 [10]. The economic benefits derive from their biological characteristics, such as antioxidants, immunostimulants, antimicrobials and anticarcinogenic [11].

Agro-industrial waste is generally a problem for processing industries, and as such, is often disposed of incorrectly in the environment, generating negative environmental impacts. These materials are sources of phytochemicals such as phenolic compounds, flavonoids, pigments, among others, which have high antioxidant activity [12].

Conventional techniques for extracting phenolic compounds, such as reflux and maceration, require more time and high temperatures, which can degrade the compounds [13]. Ultrasound-assisted extraction, studied as an alternative, offers advantages such as shorter time, lower temperature and greater efficiency in extracting active compounds [14,15]. Ethanol, considered environmentally friendly and approved by the FDA, is an effective solvent in the extraction of phenolic compounds and is often used in mixtures with water [16].

The use of ultrasound in extraction promotes cavitation, a phenomenon in which waves generate cycles of compression and expansion that rupture cell walls, facilitating solvent entry and improving mass transfer, which increases the release of target compounds. This ultrasonic energy can be applied via a water bath or directly into the sample. Temperature control is essential to protect heat-sensitive substances. Considered an economical technique, ultrasound requires simple equipment, lower initial costs, and reduces extraction time [17, 18].

Using Design of Experiments (DOE) to optimize processes

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is a promising tool, as this method is capable of controlling variables that influence this process, allowing the clear identification of the effects of each factor and the possible interactions between them. By designing experiments systematically, it is possible to optimize a process or product, maximize yield or reduce costs, without the need to perform many tests [19, 20].

In this context, this work aimed to optimize the ultrasound-assisted extraction of phenolic compounds from pracaxi residue through a 2^3 experimental design with Response Surface Methodology (RSM), where the effects of the operational variables were evaluated: extraction time (min) solid-liquid ratio (% m/v) and ethanol concentration (% v/v), to maximize the extraction of the compounds of interest, as well as the effect of the application of ultrasound in the process.

Materials and methods

Obtaining the sample

The biomass (residue) of pracaxi (Figure 1, A2D84C7 genetic heritage registry sisgen), kindly provided by the company AmazonOil (located in Ananideua-PA), was used. The biomass was ground in ball mills at the company itself and stored in polyethylene bags. Upon arrival at the Animal Nutrition Laboratory (LABNUTAN), the samples were organized, distributed in smaller polyethylene bags (around 1 kg each) and stored at room temperature (since the moisture content of the material was less than 10% at the time of analysis and all analyses).

Figure 1: Pracaxi biomass.

Reagents and equipment used in chemical and instrumental analyses

Reagent

The following reagents were used: n-hexane 98% (Merck, CAS 110-54-3), absolute ethanol (Merck, CAS 64-17-5), HPLC-grade acetonitrile (Sigma-Aldrich, CAS 75-05-8), Folin-Ciocalteu phenolic reagent (Sigma-Aldrich, CAS 631-69-6), gallic acid standard (Sigma-Aldrich, CAS 149-91-7), quercetin standard (Sigma-Aldrich, CAS 117-39-5), phosphoric acid (Sigma-Aldrich, CAS 7664-38-2), aluminum chloride (Sigma-Aldrich, CAS 7446-70-0), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich, CAS 30931-67-0) and standard Trolox (Sigma-Aldrich, CAS 53188-07-1).

Equipment

- Drying and Sterilization Ovens (Model SP-100 – SPLABOR).
- Muffle Furnace (Model- SP 1200-SPLABOR).
- Nitrogen/Protein Distiller (Model MA-036 – MARCONI).
- Lipids Extractor (Model XT10- ANKON).
- Ultrasonic tank 3.8 L frequency - 40Khz (Model - SSBu-P- Solidsteel).
- Centrifuge (Model 3K30 - SIGMA).
- Rotary Vacuum Evaporator (Model MA-120-MARCONI).
- Freeze dryer (Model L108- LIOTOP).
- Spectrophotometer (Thermo Fisher Scientific Oy, Multiskan Go-SN-1530-8001397, Finland).
- High liquid chromatograph (Shimadzu, Japan) with an LC10AT controller, a DGU-14A pump coupled to a Rheodyne manual injector, and a UV/vis detector with SPD-M10A VP diode array was used.

Physicochemical characterization of the residue

The biomasses were characterized following the protocol of the Official Methods of Analysis of the Association of Official Analytical Chemists [21], where the following analyses were determined: moisture, no. 925.09 of AOAC [21], lipids no. 925.38 of AOAC [21], crude protein no. 920.87 of AOAC [21], ash no. 923.03, AOAC [21]. Analyses were performed in triplicate, all data were tabulated, means and standard deviations were calculated, considering a coefficient of variation of up to 10%; data were expressed on a dry basis in g 100/g of pracaxi residue matter.

Biomass degreasing

To avoid interference from fat, the samples were decreased with n-hexane at a ratio of 1:6 (mass: volume or m/v) for 6 h under constant agitation, at room temperature [22]. After this time, samples were crushed, sieved and stored in plastic bags and kept at room temperature for bioactive compounds extraction analyses.

Optimization of the extraction of bioactive compounds

Firstly, preliminary tests were carried out to choose the minimum and maximum extraction points; the extraction was based on the work of [23] with modifications.

The 2^3 central composite design was used with 3 independent variables, namely extraction time (min), solid-liquid ratio percentage (m/v) and solvent or ethanol concentration percentage (v/v) with 8 trials plus 6 axial points and 3 repetitions at the central point, constituting a total of 17 trials. The dependent variables (responses) were the content of total phenolic compounds, total flavonoids and antioxidant activity (ABTS assay). Table 1 presents the levels of each study variable.

Table 1: The levels of each study variable.

Variables	**Levels**				
Code	-α 1,68	-1	0	+1	+α 1,68
$\mathbf{X_1}$	0.34	15	37.5	60	75.34
$\mathbf{X_2}$	0.1	5	1.25	2	2.5
$\mathbf{X_3}$	0.01	0.5	32.5	60	78.75

Note: X_1 = Extraction time (min); X_2 = Solid-liquid ratio (%) m/v); X_3 = Solvent concentration ((%) v/v).

For the tests, the number of defatted samples was weighed with the solid-liquid ratio, ethanol concentration and residence time according to the experimental design. The tests were performed in 250 mL Schott flasks subjected to a Solid Steel 3.8L ultrasonic tank, 40 kHz, 35°C. After completion of the reactions, the liquid fraction was separated from the solid fraction by centrifugation in SIGMA centrifugation at 10,000 g in room temperature, which was discarded, the liquid fraction was stored in amber bottles to be analyzed in terms of total phenolic compounds (TPC), total flavonoids (TF) and antioxidant activity by ABTS.

Response surface methodology

Response Surface Methodology (RSM) is a statistical and mathematical approach aimed at the analysis and optimization of processes with multiple independent variables influencing a response variable. This methodology, widely used in engineering, especially in the chemical area, allows the modeling and understanding of complex systems through response functions, being a valuable tool in experimental studies [24].

In this work, RSM was performed according to the Equations I and II [25][26]:

$$Y = \emptyset(x_1, x_2, \dots, x_k) \pm e_r \quad (I)$$

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i + \sum b_{ij} x_i x_j + e \quad (II)$$

Where Y represents the estimated response, b_0 is the constant term, while b_i, b_{ii} and b_{ij} correspond to the linear, quadratic and interaction coefficients, respectively.

e: is the experimental error or residual, which represents the difference between the observed value and the value predicted by the model. It incorporates variations not explained by the model variables.

Desirability function for multi-response analysis

Optimization processes by the desirability function (Df) is a widely used technique that integrates DF analysis with design of experiments (DOE). It is a practical, consolidated and easy-to-apply approach, not restricted to a specific type of optimization problem. This methodology is especially useful when one wants to optimize several responses with different objectives simultaneously. Its main objective is to find the best set of input variables that allows all responses to come as close as possible to the desired values or established goals [26].

Since this work deals with multiple responses (in this case, 5), this work uses the calculation of desirability, different from the authors original approach. The desirability function was proposed by Derringer and Suich [27] and aims to find a compromise condition where all responses are within a region accepted as desirable. Thus, the original responses are coded between 0 (undesirable condition) and 1 (desirable condition).

The desirability function aims to find a compromise condition where all responses are within a region accepted as desirable. In this way, the original responses are coded between 0 (undesirable condition) and 100% (desirable condition) [27].

Analysis of bioactive compounds

Total phenolic compounds (TPC)

It was performed according to the methodology proposed by Singleton & Rossi [28], modified for microplates, where a reaction is performed in a 96-well microplate, in which 25 µL of extract will be inserted, followed by the addition of 125 µL of Folin-Ciocalteu reagent at 10% (v/v), leaving the reaction to occur for 2 min. After this time, 100 mL of sodium carbonate at 7.5% (m/v) will be added, and the plate will be placed in the spectrophotometer (Thermo Fisher Scientific Oy, Multiskan Go-SN-1530-8001397, Finland) with shaking for 30 seconds followed by rest for 30 minutes. The absorbance reading was performed at a wavelength of

765 nm. The concentration of CFT in the extracts was determined from the equation of the straight line obtained in the standard curve of gallic acid (Sigma, 99% purity) (y=0.0059x+0.0929, R^2 = 0.9996) and expressed in mg GAE/g of sample.

Total flavonoids (TF)

FT contents in the extracts were quantified according to the method described by [29], with modifications. 115 µL of sample extract and 115 µL of 2% $AlCl_3$ solution were added. The plate was then placed in the spectrophotometer for shaking for 30 seconds and then left to stand for 10 minutes. The absorbance was then measured at 425 nm. The total flavonoid contents were calculated from a quercetin standard curve (y=0.02x-0.0047, R^2 = 0.9987) and expressed in (mg QE/100g).

Antioxidant activity by the ABTS method

The antioxidant capacity of the samples against the free radical ABTS•+ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was evaluated according to the methodology proposed by Rufino et al. [30]. The ABTS•+ cation was produced by the reaction of a stock solution of 7 mM ABTS (>98%, Sigma-Aldrich, Brazil) with 140 mM potassium persulfate (Neon, Brazil). The reactions were performed by transferring 30 µL of sample to test tubes containing 3.0 mL of the ABTS•+ radical, and the reading will be taken at 734 nm after 6 minutes of mixing. Quantification was performed using Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%, Sigma, Brazil) to construct the analytical curve (y = -0.0002x+0.7055, R^2 = 0.9902). From the equation of the straight line, the calculation was performed and expressed in micromoles of Trolox equivalent per gram of extract (µM Trolox equivalent/g of sample).

Identification of phenolic compounds by high-performance liquid chromatography (HPLC)

A liquid chromatograph (Shimadzu, Japan) with an LC10AT controller, a DGU-14A pump coupled to a Rheodyne manual injector, and a UV/vis detector with SPD-M10A VP diode array was used. The compounds will be analyzed at a wavelength of 254 to 280 nm. The software used was Real Time. The column used was the C18 XBridge BEH of 150 x 4.6 mm and 3.5 µm particles (Waters - Ireland) in reverse phase. The injection volume was 20 µL, and the mobile phase gradient was: Solvent A, 0.1% TFA in MilliQ water; Solvent B, 0.1% TFA in 90% acetonitrile and 10% MilliQ water. Two HPLC-grade standard solutions of gallic acid and quercetin (Sigma-Aldrich) were injected to suggest the identification of the extract peaks. The total running time was 35 minutes. Our research group adapted this methodology only to suggest the molecules of phenolic compounds or flavonoids present in the analyzed extracts.

Statistical analysis

Responses of the experimental design (total phenolic compounds, total flavonoids and antioxidant activity) underwent analysis of variance, with the F regression test and F lack of adjustment test (considering the pure error) being performed to validate these data and generate response surfaces.

Analyses were performed using Statistic software (Version 7) and the confidence level adopted was 90% ($p < 0.1$).

Results and discussion

Physicochemical characterization of the residue

Table 2 presents the data on the centesimal composition of pracaxi biomass.

Table 2: Centesimal composition of pracaxi biomass.

Determination (g/ 100 g)	Pracaxi biomass	Variation coeficient (%)
Moisture	13.12±0.26	1.98
Ash	3.5±0.05	1.42
Lipids	18.03±0.21	1.16
Proteins	12.1±0.14	1.60
Carbohydrates	66.37	-

Pracaxi biomass presented an interesting composition, with 13.12% moisture, 3.5% ash, 18.03% lipids, 12.1% proteins and 66.37% carbohydrates. With a high content of lipids and carbohydrates, it is a promising source for industrial applications, such as the production of oils, cosmetics and bioenergy. In addition, the low ash content and the good number of proteins and carbohydrates suggest that it is a quality biomass with nutritional and functional potential. The results found in this study were close to those found by Fonseca and Pereira [31], who found values of 21±0.2 lipids, 21±0.1 proteins, 12±0.5 fibers, 2.5±0.1 ash, 29±0.1 carbohydrates and 14±0.07 moisture, with a significant difference in the protein and carbohydrate contents when compared to this research.

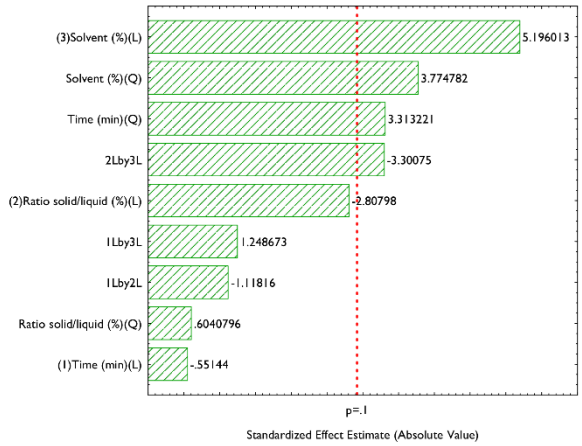
Optimization of the extraction of bioactive compounds

Figure 2 shows the Pareto chart generated for the bioactive compounds and the influence of the studied factors (independent variables) on the responses (dependent variables),

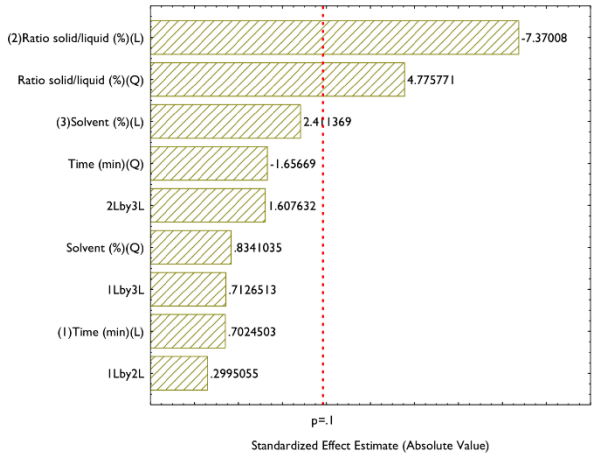
TPC (Figure 2a), TF (Figure 2b) and Antioxidant Activity (AA) by ABTS (Figure 2c).

Figure 2a shows that the significant effects (p≤0.1) that influenced TPC extraction were, respectively, the linear (L) and quadratic (Q) solvent percentage, quadratic extraction time (Q) and solid-liquid interaction with solvent concentration. When a linear effect is positive, it indicates a direct and proportional relationship with the independent variable and the response (dependent variable); when it is positive, it indicates that the increase of this variable leads to a rise in TPC concentration. In this work the TPC concentration had a significant and positive quadratic variable, this suggests that there is a non-linear relationship between the independent variable and the response, showing that there is the presence of a maximum region, and that an excessive increase or decrease of the independent variable can decrease TPC extraction, a positive quadratic value indicates the presence of an upward concavity in the graph and the non-linear relationship in the mathematical model.

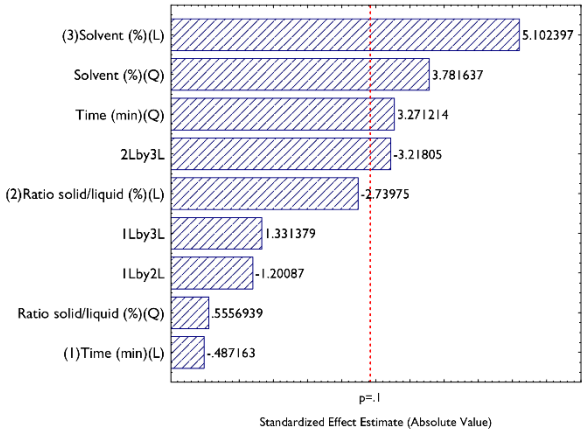
For Figure 2b, it was observed that only the linear effects of solid-liquid ratio (L) and solvent concentration (L) had a significant impact on the response. In addition to this, there was also significance of the quadratic solid-liquid ratio effect, indicating a positive value and the presence of a maximum region for this parameter as well. The impact of the solid-liquid ratio (L) had a negative value, indicating that with the increase in mass in the solid-liquid ratio, there is a decrease in response. The effect of the solid-liquid ratio was not significant for TPC, which suggests that specific factors are more unfavorable for this subgroup of bioactive compounds.



(a)



(b)



(c)

Figure 2: Pareto chart for TPC (a); TF (b) and AA (c).

Finally, Figure 2c shows the effects of the variables on the AA response by ABTS, indicating that the linear solvent (L), quadratic solvent (Q), quadratic extraction time (Q) and the solid-liquid and solvent interaction, both linear, had a value of p≤0.1, which indicates that their effects were significant in the AA increase responses. If there is an increase in the solvent, there is an increase in the AA response. However, due to the presence of the quadratic region, there is a maximum production and also a non-linear relationship that must be considered.

Guerra et al. [15] studied the effect of ultrasound-assisted extraction of phenolic compounds from the peel of Tommy Atkins mangoes, studying ethanol concentrations of 30, 50 and 70% (v/v), temperatures of 40, 50 and 60 °C, and extraction times of 30, 40 and 50 min through a Box-Behnken experimental design. The results showed

that all variables evaluated had a positive effect on the extraction of phenolic compounds, except for the interaction between time and ethanol concentration. These authors were able to achieve concentrations of up to 20.76 mg GAE/g of sample.

The non-significant effects in the Pareto analysis were ignored and Analysis of Variance (ANOVA) (Table 3) was performed to assess whether the mathematical models can be considered significant and predictive. For this, two F tests were performed: one related to regression ($F_{regression}$) and the other related to lack of fit ($F_{lackoffit}$). The difference between them is that $F_{regression}$ assesses whether the regression model is statistically significant, that is, whether the model coefficients (independent variables) significantly explain the variation in the dependent variable. $F_{lackoffit}$ will assess the adequacy of the adjusted model to the experimental data, considering the residuals and pure errors. In this research, the analysis was made considering the pure error; it was necessary to perform both analyses to assess the relevance of mathematical models at the time of adjustment.

For a mathematical model to be considered significant and predictive, the tabulated $F_{regression}$ must be greater than the calculated F, while the calculated $F_{lackofadfit}$ must be less than the tabulated F for lack of fit.

In this context we can observe that for TCP tabulated $F_{regression}$ for a df of 4 and 12 the value was 2.48 ($p \leq 0.1$) and the calcultated $F_{regression}$ was 3.86, for the lack of fit F, the tabulated value for a df of 12 and 2 was 9.39 (calculated F = 5.40). This implies that for AA the mathematical model passed the test, being considered significant and predictive.

Also, for TF the tabulated $F_{regression}$ for a df of 2 and 14 the value was 2.73 ($p \leq 0.1$) and the calcultated $F_{regression}$ was 10.49, for the lack of fit F, the tabulated value for a df of 10 and 2 was 19.41 (calculated F = 4.02). This implies that for AA the mathematical model passed the test, being considered significant and predictive.

Regarding AA, the tabulated $F_{regression}$ for a df of 4 and 12, the value was 2.48 ($p \leqslant 0.1$), and the calculated Fregression was 3.84. For the lack of fit F, the tabulated value for a df of 10 and 2 was 9.39, and the calculated F was 5.29. This implies that for AA the mathematical model passed the test, being considered significant and predictive.

Table 3: Analysis of variance (ANOVA) and F tests for the design of experiments of ultrasound-assisted extraction of pracaxi biomass.

Source of variation	**TPC**				
Effects	**QS**	**Df**	**QM**	**Fregression**	**Flackof fit**
Regression	0.6218	4	0.1554	3.86	5.40
Residue	0.4833	12	0.0403		
Lack of fit	0.466020	10	0.046602		
Pure error	0.017267	2	0.008633		
Total	56.27	-	-		
% variation explained	98.44	-	-		
Maximum % explainable variation		-	-		
	TF				
Regression	0.0013	2	0.0007	10.49	4.02
Residue	0.0009	14	0.0001		
Lack of fit	0.000847	12	0.000071		
Pure error	0.000035	2	0.000018		
Total	0.002205	16	-		
% variation explained	59.97	-	-		
Maximum % explainable variation	98.41	-	-		
	AA				
Regression	11555.8111	4	2888.9528	3.84	5.29
Residue	9030.8882	12	752.5740		
Lack of fit	8701.95	10	870.195		
Pure error	328.93	2	164.467		
Total	20586.70	16	-		
% variation explained	56.27	-	-		
Maximum % explainable variation	98.44	-	-		

QS = quadratic sum; Df =degree of freedom; QM = quadratic mean; TPC = Total Phenolic Compound; TF = Total flavonoids and AA Antioxidant actitivity by ABTS.

The ANOVA analysis indicated that all models passed the F tests for regression analysis and lack of fit. The mathematical models were then considered significant and predictive. The mathematical models follow a polynomial equation and can be seen in the Equations. 1, 2 and 3 below in the order TPC, TF and AA, respectively.

$$TPC=0,159095+0,086953*X1^2+0,143040*X3+0,120958*X3^2-0,108432X2*X3 \quad (1)$$

$$TF= 0,011513 -0,008558*X2 + 0,006454*X2^2 \quad (2)$$

$$AA=21,8935+11,8883*X1^2+19,3863*X3+16,7622*X3^2-14,5911\ X2*X3 \quad (3)$$

*Note: X1 = Extraction time (min); X2 = Solid-liquid ratio (%) m/v); X3 = Solvent concentration (%) v/v).

To determine the optimum extraction point, the desirability function was used, as shown in Figure 3. The optimum condition determined was an extraction time of 20 min, a solid-liquid ratio of 0.7% (m/v) and an ethanol concentration of 70% (v/v), with an overall desirability value of D = 71.57%, which indicates good condition [27].

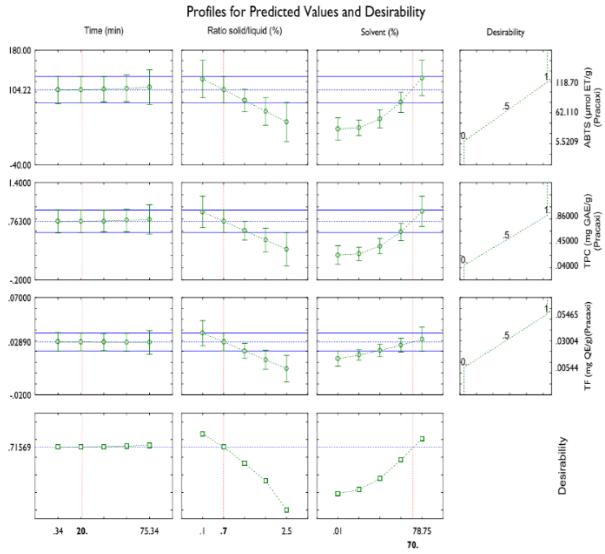


Figure 3: Profile of predicted values and desirability for the extraction efficiency of total phenolic compounds from pracaxi biomass.

After this analysis, the contour surfaces were generated for all variables studied, considering the overall desirability, which are shown in Figure 4.

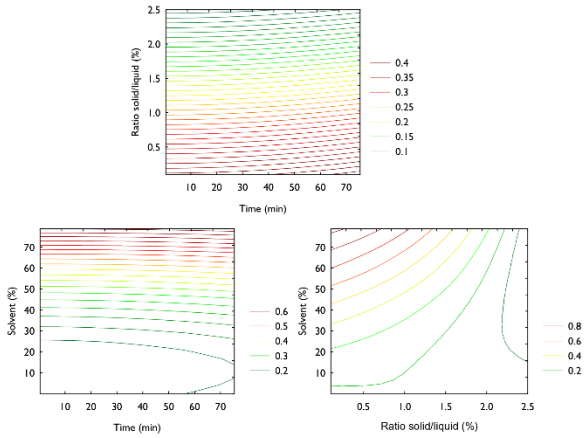


Figure 4: Response surfaces of the global desirability function (D).

According to Figure 3, it can be observed that, in general, the variables that had the most significant effect on the extraction of bioactive compounds were the solid-liquid ratio, followed by the solvent concentration and extraction time.

In this work, it was determined to achieve values close to 0.763 mg GAE/g for TPC, 0.029 mg QE/g and 104.22 µmol ET/g of sample. These data were obtained through the mathematical model of optimization by the desirability tool. This condition was performed at the laboratory where the following experimental values were obtained: 0.85 mg GAE/g; 0.028 mg QE/g and 92 µmol ET/g, with error values of: 10.23%; 3.57% and 13.28%. Indicating that the model obtained can be considered satisfactory to explore the potential of pracaxi biomass.

Another experiment was carried out under the same conditions, but using 70% (v/v) methanol and a thermostatic bath (Quimis Dubnoff), subjecting the reaction for one hour at a temperature of 50°C, and then quantifying only the TPC, obtaining values of 0.90 mg/g±15.01, indicating that the use of ethanol with assisted ultrasound is an efficient technique for the extraction of bioactive compounds from pracaxi, being economically viable because it uses less time, more mass and a green solvent, which will not cause damage to the environment, in addition to having the possibility of valuing a residue from the Amazon, contributing to the circular economy.

Comparing these findings with the literature, Mohammadnezhad et al. [32] employed supercritical fluid extraction (SFE) with CO_2 to obtain lipid fractions from pracaxi nuts. Their results indicated that temperature significantly influenced the extraction yield, total phenolic content, and bioactivity. However, they noted that higher extraction yields did not always correlate with greater bioactivity. Under optimized conditions, their extracts contained 91.9 ± 0.9 mg GAE/g (80% EtOH) and 103.9 ± 0.5 mg GAE/g (12.5% EtOH) for TPC, with TF values of 6.6 ± 0.2 mg QE/g and 7.0 ± 0.1 mg QE/g, respectively.

Additionally, Eberhart et al. (2023) [33] analyzed the total phenolic composition in pracaxi oil, reporting a value of 67.43 mg GAE/kg. Teixeira et al. (2020) [34] found phenolic contents ranging from 31.92 to 54.05 mg GAE/kg in pracaxi oil. Such variations can be attributed to environmental factors, including climate, geographic location, humidity, and harvest time, as well as differences in extraction techniques, which can influence the final composition of bioactive compounds [34].

While the values reported in the literature for pracaxi-based extracts are generally higher than those obtained in the present study, it is important to consider differences in extraction methodologies and sample types. The ultrasound-assisted extraction with ethanol used here offers advantages in terms of process efficiency, environmental sustainability, and economic viability, reinforcing its potential for large-scale application.

Chromatogram by high-performance liquid chromatography (HPLC)

Figure 5 displays the results of a High-Performance Liquid Chromatography (HPLC) analysis, showing the separation of a mixture's components over time. Each peak represents a different compound, with the largest peak likely being Catechin.

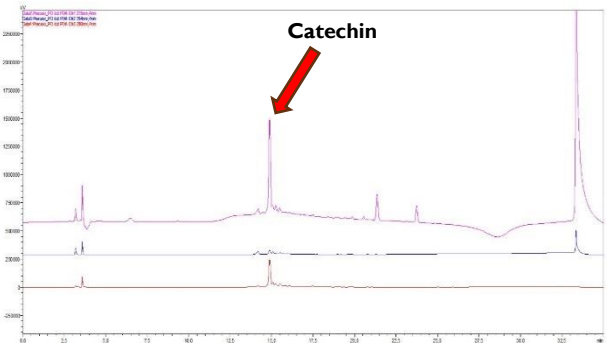


Figure 5: Chromatogram of the optimized condition for phenolic compounds of pracaxi.

The sharp peak for catechin indicates a clean separation, and its height suggests a significant amount in the sample. To further confirm the presence of catechin, additional analytical techniques can be employed, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR). Catechin is a type of flavanol, known for its potential health benefits, including protection against cell damage, heart disease, cancer, diabetes, and neurodegenerative diseases. It may also help improve brain health. Further research is ongoing to confirm the benefits of catechin fully, but it shows promise as a natural compound with positive effects on health.

Conclusions

This study demonstrated that pracaxi biomass has a significant number of bioactive compounds, such as phenolic compounds and flavonoids, which have antioxidant activity. The optimization of the ultrasound-assisted extraction process, using the 2^3 experimental design with Response Surface Methodology (RSM) and desirability function, allowed us to determine the best conditions for extracting these compounds, with an extraction time of 20 min, a solid-liquid ratio of 0.7% (m/v) and an ethanol concentration of 70% (v/v). The results obtained in this study show that pracaxi biomass is a promising source of bioactive compounds for the food, pharmaceutical and cosmetic industries, and that ultrasound-assisted extraction is an efficient and sustainable technique for obtaining these compounds. The prospects for this work are to perform a mass spectroscopy analysis for more rigorous identification of the bioactive molecules present in these extracts, and thus contribute to the bioeconomy and development of the Amazon.

List of abbreviations

AA: Antioxidant Activity
ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA: Analysis of Variance
DOE: Design of Experiments
FDA: Food and Drug Administration
GAE: Gallic Acid Equivalent
HPLC: High-performance Liquid Chromatography
MS: Mass Spectrometry
NMR: Nuclear Magnetic Resonance
QE: Quercetin Equivalent
RSM: Response Surface Methodology
TF: Total Flavonoids
TFA: Trifluoroacetic Acid
TPC: Total Phenolic Compounds

Author Contributions

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Availability of Data and Materials

Not applicable.

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Conflicts of interest

The authors declare there is no conflict of interest for this work.

Figures and Tables Originality

The research itself authors all the Figures and Tables in this work.

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